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Acellular Vascular Grafts Generated from Collagen and Elastin Analogues

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Abstract

Tissue engineered vascular grafts require long fabrication times, in part, due to the requirement of cells from a variety of cell sources to produce a robust load bearing, extracellular matrix. Herein, we propose a design strategy for the fabrication of tubular conduits comprised of collagen fiber networks and elastin-like protein polymers to mimic native tissue structure and function. Dense fibrillar collagen networks exhibited an ultimate tensile strength (UTS) of 0.71 ± 0.06 MPa, strain to failure of $37.1 \pm 2.2\%$, and Young's modulus of 2.09 ± 0.42 MPa, comparing favorably to an UTS and a Young's modulus for native blood vessels of 1.4 – 11.1 MPa and 1.5 ± 0.3 MPa, respectively. Resilience, a measure of recovered energy during unloading of matrices, demonstrated that $58.9 \pm 4.4\%$ of the energy was recovered during loading-unloading cycles. Rapid fabrication of multilayer tubular conduits with maintenance of native collagen ultrastructure was achieved with internal diameters ranging between 1 to 4 mm. Compliance and burst pressures exceeded $2.7 \pm 0.3\%/100$ mmHg and 830 ± 131 mmHg, respectively, with a significant reduction in observed platelet adherence as compared to ePTFE ($6.8 \pm 0.05 \times 10^5$ vs. $62 \pm 0.05 \times 10^5$ platelets/mm², $p < 0.01$). Using a rat aortic interposition model, early in vivo responses were evaluated at 2 weeks via Doppler ultrasound and CT angiography with immunohistochemistry confirming a limited early inflammatory response ($n=8$). Engineered collagen-elastin composites represent a promising strategy for fabricating synthetic tissues with defined extracellular matrix content, composition, and architecture.

Keywords

Elastin-mimetic protein; collagen fiber; biofabrication; vascular graft

INTRODUCTION

The design of a tissue engineered vascular graft to replace diseased arteries requires consideration of mechanical, biological, and clinical factors that influence behavior in vitro and in vivo [1–3]. While much progress has been made in determining the key factors that contribute to the eventual success of a graft, to date, widespread acceptance of a tissue engineered conduit as an acceptable alternative to either an autologous artery or vein has yet to be achieved. Mechanical requirements for an arterial substitute includes sufficient burst pressure to prevent catastrophic failure and long-term fatigue resistance; compliance, which approximates that of native vessels to prevent mechanical mismatch; and suitable suture retention to permit implantation in a manner that tolerates hydrodynamic and mechanical forces at the anastomoses. Biological and clinical considerations have focused on the generation of a non-fouling luminal surface to prevent thrombosis and the minimization of inflammatory events either due to surgery or graft properties that may contribute to early or late graft failure [1, 4].

Several groups have demonstrated the efficacy of various strategies that vary from modification of existing ePTFE/DacronTM grafts, acellular or cellularized constructs, to de novo engineering of tissue substitutes that mimic native vessels [5]. Tissue engineered blood vessels derived from cell-sheet tissue engineering and degradable synthetic polymer scaffolding has demonstrated early clinical success and continued progress with several additional systems suggest that these technologies will continue to evolve [3, 6, 7]. We believe that clinical success will ultimately require utilizing a “bottom-up” approach where recapitulation of the fundamental features of the vascular wall, incorporation of key elements that obviate thrombosis and acute graft failure, and potentially the addition of a cellular component to provide a means for self-repair and other functional properties required for long-term graft patency [8–10]. The strategy reported herein does not rely on the process of seeded cells to produce ECM or bioreactor conditioning of cell containing constructs, which typically requires weeks to months of process time and mandates either the use of autologous cells or the removal of allogeneic cells [11–13]. We believe that avoiding these steps will shorten fabrication time, enhance tissue integrity, and improve biological responses after in vivo implantation.

Our lab and others have recently synthesized and characterized a series of elastin-like protein polymers that consist of sequentially repeated amino acid blocks [14–17]. With the ability to easily modify peptide chain length, consensus repeat sequence, and introduce additional oligopeptide units, protein polymers can be produced with enhanced biological, thermodynamic, and mechanical properties. We have designed a series of elastin-like polypeptides that can be fabricated as films or other geometrical constructs, have robust mechanical properties, a high degree of resilience, minimal thrombogenicity, and long-term stability in vivo [18–26].

Collagen, a vital component of the extracellular matrix (ECM), is required both as a load-bearing element and as a mediator of local biological responses. Electrospinning, casting gels, and wet spinning have been the mainstay for large-scale production of collagen matrices for tissue engineering. Although much progress has been made in the field of electrospinning, the use of organic solvents leads to collagen denaturation [27]. Likewise, the lack of mechanical integrity of collagen hydrogels precludes their use as a structural component. The objective of this study was to develop a strategy for processing dense collagen fiber networks, embedded with an elastin-like protein matrix, to function as a mechanical and tissue-mimetic analog for fabrication of an arterial substitute. Composite structures were fabricated with defined composition and microarchitecture with preservation of native collagen structure.

This report describes the rapid fabrication of protein-based matrices of high strength and stiffness approximating native tissue. Mechanical characterization of non-crosslinked matrices illustrates the potential to modulate and tailor mechanical strength for a variety of vascular and other soft tissue engineering applications. We hypothesize that these composites may prove useful for blood contacting applications given their hemocompatibility and in vivo stability.

MATERIALS and METHODS

Isolation and purification of monomeric Type I collagen

Monomeric Type I rat tail tendon collagen was obtained by acid extraction from Sprague-Dawley rats (Pel-Freez Biologicals, Rogers, AR) following a procedure adapted from Silver and Trelstad [28]. Briefly, rat tail tendons were extracted with the aid of autoclaved pliers and dissolved in 10 mM HCl for 4 h at 25°C to dissolve the proteinaceous components. Insoluble tissue and other contaminants were removed by centrifugation at 30,000 g at 4°C for 30 mins with subsequent vacuum filtration through 20 µm, 0.45 µm, and 0.2 µm filters. The sterile filtered collagen in HCl was precipitated from solution by adding NaCl to a final concentration of 0.7 M. The precipitated collagen was pelleted by centrifugation, redissolved in 10 mM HCl, and dialyzed first against 20 mM phosphate buffer at room temperature, then at 4°C against 10 mM HCl at 4°C, and finally against deionized water at 4°C. The collagen was then frozen and lyophilized till use.

Synthesis of a recombinant elastin-like protein polymer (ELP)

Development and production of the ELP, LysB10, has been described elsewhere [24]. Briefly, a triblock amphiphilic copolymer was designed to contain hydrophobic endblocks and a hydrophilic midblock. The 75kDa endblock is comprised of 33 repeats of the pentapeptide sequence [IPAVG]₅ and the 58 kDa midblock comprised of 28 repeats of the sequence [(VPGAG)₂VPGE(VPGAG)₂]. Flanking both the hydrophobic, plastic endblocks, and the hydrophilic, elastic midblock, were crosslinkable amino acid sequences [KAAK], which allows for amine-based crosslinking. Subsequent to expression in *E. coli*, protein was extracted and purified using hot/cold centrifugation cycles and nucleic acid removal. Protein solutions were then dialyzed against water and lyophilized.

Production of dense collagen networks

Monomeric rat tail tendon collagen and Lys-B10 were dissolved in 10mM HCl at a concentrations of 2.5 mg/mL. Solutions were neutralized using a gelation buffer, GB, (4.14 mg/mL monobasic sodium phosphate, 12.1 mg/mL dibasic sodium phosphate, 6.86 mg/mL TES (N-tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid sodium salt, 7.89 mg/mL sodium chloride, pH 8.0) at 4°C and were poured immediately into rectangular molds (10 × 7 × 0.4 cm) for 24 h. Gels were subsequently placed in a fiber incubation buffer, FIB, (7.89 mg/mL sodium chloride, 4.26 mg/mL dibasic sodium phosphate, 10 mM Tris, pH 7.4) at 37°C for 48 h to promote collagen fibrillogenesis [29]. Gels were then dried at room temperature under a steady air stream.

Imaging of composite architecture

Optical microscopy, fluorescence microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were used to analyze the collagen micro and ultrastructure prior to and after embedding in elastin. For SEM studies, dry collagen mats were hydrated in water for 24 h and dehydrated in serial exchanges of ethanol-water mixtures from 30%–100%. The samples were then critical point dried (Auto Samdri 815 Series A, Tousimis, Rockville, MD), sputter coated with 6 nm of Pt/Pd (208HR Cressington,

Watford, England), and imaged at an accelerating voltage of 10 keV using a field emission scanning electron microscope (Zeiss Supra 55 FE-SEM, Center for Nanoscale Systems, Harvard University). For TEM studies, samples in PBS were washed in 0.1 M cacodylate buffer and fixed in glutaraldehyde. After washing in water, samples were partially dehydrated in ethanol and stained with uranyl acetate. Samples were then fully dehydrated in ethanol, embedded in resin, and polymerized. Ultrathin (60 – 80 nm) sections were cut using a RMC MT-7000 ultramicrotome (Boeckeler, Tucson, AZ). Post-staining with uranyl acetate and lead citrate was followed by imaging using a JOEL JEM-1400 TEM (JOEL, Tokyo, Japan) at 90 kV.

Fabrication collagen-elastin nanofibrous grafts

Lys-B10, dissolved in molecular grade water at 4°C at a concentration of 100 mg/mL, was used to embed acellular collagen matrices in a sandwich molding setup (Fig. 1). Briefly, collagen matrices were dried on 0.45 µm filter membranes (Millipore, MA), and cooled to 4°C. Plastic shims (height 72.6 µm, Precision Brand, IL) were placed around the collagen sheet. Cold elastin was spread on top of the collagen matrix and a capping glass layer was added for 20 mins. The setup was warmed to 25°C to allow the liquid elastin to gel. The collagen-elastin composites were then removed from the glass support, the long sheets were rolled on 1.3 mm or 4 mm ID stainless steel mandrels, rotated at 60 rpm at 4°C for 10 min to allow the elastin to undergo a phase transition to a liquid state, and then rotated at 60 rpm at 25°C for 5 min to gel the elastin into one continuous layer.

Mechanical testing of composites

Planar constructs—Collagen sheets were cut using a dog-bone punch (13 mm gage length), mounted onto a Dynamic Mechanical Thermal Analyzer V (DMTA V, Rheometric Scientific, Piscataway, NJ), and immersed in PBS at 37°C. Samples were preconditioned 15 times to 66% of the average maximum failure strain of initial test samples, and then tested to failure at 5 mm/min. A total of four samples were tested for each group. Thickness of hydrated samples was measured using optical microscopy. Young's modulus was determined from the slope of the last 4% of the stress-strain curve, in addition to ultimate tensile strength (UTS) and strain at failure.

Tubular constructs—Pressure-diameter testing to determine compliance and burst pressure of constructs was performed, as previously reported [29]. Briefly, tubular collagen-elastin composites were mounted vertically, via Luer-lock connectors with a 5 g axial weight in PBS at 37°C. Grafts were inflated at a rate of 10 mmHg/s, monitored using a pressure transducer (WIKA, Lawrenceville, GA), and videographed for distention, using a CCD camera. An edge detection program was written in MATLAB to identify and quantify radial distension of grafts based on the outer diameter and correlated to pressure readings. Compliance was determined as the percent difference in outer diameter at systole and diastole, divided by the pressure difference and initial outer diameter. The burst pressure, at which the graft started to leak, was also determined for 4 mm and 1.3 mm diameter grafts (n = 4). Suture retention strength was determined by cutting 4 mm × 4 mm square sections from longitudinal sections of the graft wall (n = 4). A 4-0 FS-2 Prolene™ suture (Ethicon, Inc.) was thrown through the middle of the square segment and pulled in the longitudinal direction using a DMTA (Rheometric Scientific). Wall thickness measurements were made on four representative cross-sections of each graft. Image analysis using Adobe Photoshop allowed measurement of inner and outer diameters and wall thickness (n = 4).

Platelet adhesion assay

Platelet adhesion and morphology was determined by incubation of human platelets on grafts and control surfaces, as described [30, 31]. All methods were approved by the Institutional Review Board of the Committee on Clinical Investigations, Beth Israel Deaconess Medical Center. Blood from healthy volunteers was drawn via venipuncture into buffered citrated sodium (Citate Tubes, BD Biosciences, Franklin Lakes, NJ). To isolate platelet rich plasma (PRP), blood was centrifuged at 200 g for 15 minutes at room temperature. PRP was withdrawn from the upper fraction of tubes with care not to aspirate the red blood cell component. The number of platelets per mL of PRP was determined by flow cytometry, 260 million platelets/mL. PRP was immediately added to different surfaces, including glass (positive control), ePTFE, collagen (structural material of the graft), elastin (blood contacting surface of the graft), and graft surfaces (n=4). PRP was incubated on surfaces for 30 min in a humidified 37°C incubator, aspirated, and surfaces washed three times with 1x PBS to remove non-adherent platelets. Adherent platelets were quantified using a lactate dehydrogenase cytotoxicity kit (LDH, Roche Diagnostics, Indianapolis, IN). Briefly, kit lysis buffer was added to lyse adherent platelets for 30 min. A colorimetric LDH quantification substrate was added for 30 min at room temperature, as per manufacturer's instructions. Absorbance of 450 nm light was measured and compared to a standard curve of known platelet concentrations. For determination of platelet morphology, samples were incubated with PRP and washed, adherent platelets on surfaces were fixed in 2.5% glutaraldehyde in PBS for 2.5 hr, ethanol dehydrated and critical point dried (Auto Samdri 815 Series A, Tousimis, Rockville, MD). Samples were then sputter coated with 6 nm of Pt/Pd (208HR Cressington, Watford, England) and imaged at an accelerating voltage of 10 keV using a field emission scanning electron microscope (Zeiss Supra 55 FE-SEM, Center for Nanoscale Systems, Harvard University).

Implantation of grafts in rat aortic interposition model

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Beth Israel Deaconess Medical Center. Female Sprague-Dawley rats (275–300g, Charles River Labs, Wilmington, MA) were anesthetized using isoflurane (2% for induction and 1% for maintenance), shaved, sterilely prepped, and placed on a heating mat at 37°C. A vertical midline abdominal incision was made to expose the infrarenal aorta. No pre-, peri- or post-operative medication was used. The proximal and distal aorta were clamped using microclamps and a 5 mm segment was resected and replaced with an acellular graft (1.3 mm i.d., 1.5 mm o.d.), soaked in 100 U/mL heparin overnight, using eight to ten interrupted sutures (10-0 Prolene™). The abdominal incision was closed with 3-0 Prolene™ for the fascia and muscular layers, and 4-0 Prolene™ subcuticular suture for skin. Subsequent to determining the acute thrombotic response, 1 day (n=4), graft stability was determined, 14 days (n=8) [32, 33].

Evaluation of graft performance

Histological analysis—At 14 days, rats were anesthetized (2.5% isoflurane induction, 1.5% isoflurane maintenance) and the thoracic cavity exposed. Whole body fixation was performed. Briefly, an 18 gauge needle was introduced into the left ventricle, and the animal was exsanguinated using 200 mL of saline and perfusion fixed using 200 mL of 10% buffered formalin. Samples were then processed for histology, paraffin embedded, and sectioned at 5 µm thickness. Evaluation of the ECM was determined using Masson's Trichrome, cellular infiltrate by Hemotoxylin and Eosin staining, endothelial cell staining performed using vWF and macrophage infiltration by CD68 staining (Abcam, Cambridge, MA).

Ultrasound and Computed tomography angiography (CTA)—Ultrasound was performed at 1 and 2 week time points prior using a Vevo 2100 ultrasound platform (VisualSonics, Toronto, Ontario, Canada). Color Doppler was used to determine blood flow and the pulse wave compared to aged matched rats.

CTA was performed for 3-dimensional reconstruction and evaluation of patency of the implanted grafts. At the terminal 2-week time point, four rats were anesthetized (2.5% isoflurane induction, 1.5% isoflurane maintenance) sterilely prepped and a sternotomy performed to expose the thoracic cavity. To facilitate acquisition of images, whole body exsanguination and fixation were performed and a radiopaque agent (Omnipaque, GE Healthcare, Milwaukee, WI) administered. Images were acquired using a NanoSPECT (Bioscan, Washington DC) and processed using InVivoScope (Bioscan, Washington DC).

Statistical Analysis

Mean and standard deviation were obtained for all measurements. Comparisons were made using the Student's *t* test for paired data, ANOVA for multiple comparisons, with Tukey post hoc analysis for parametric data. Nonparametric tests were carried out using the Kruskal-Wallis ANOVA, with Dunn's post hoc analysis, as indicated. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Development of collagen fiber matrices with soft tissue-matching properties

Collagen fiber networks exhibited strengths on the order of 10^5 – 10^7 Pascals, comparing favorably to traditional collagen hydrogels or elastin networks [34]. Prior to embedding with ELP, dense fibrillar collagen networks exhibited an ultimate tensile strength (UTS) of 0.71 ± 0.06 MPa, strain to failure of $37.1 \pm 2.2\%$, and Young's modulus of 2.09 ± 0.42 MPa. Subsequent to embedding with ELP mechanical properties did not change significantly. Planar composites had an UTS of 0.60 ± 0.09 MPa, strain to failure of $38.5 \pm 4.5\%$, and a Young's modulus of 1.55 ± 0.38 MPa. These mechanical properties compare favorably to the UTS and Young's modulus for native blood vessels of 1.4 – 11.1 MPa and 1.5 ± 0.3 MPa, respectively. Resilience, a measure of recovered energy during unloading of matrices, demonstrated that $58.9 \pm 4.4\%$ (collagen sheet) and $56.8 \pm 5.4\%$ (ELP embedded collagen sheet) of the energy is recovered during loading-unloading cycles.

Biomimetic vascular grafts with mechanical matching to native vasculature

The compliance of grafts with internal diameters of 1.3 mm and 4 mm was $2.7 \pm 0.3\%/100$ mmHg, and $2.9 \pm 0.2\%/100$ mmHg, respectively, which was similar that of native saphenous vein ($0.7 - 2.6\%/100$ mmHg) (Table 1) [35, 36]. Burst pressures were 830 ± 131 mmHg for 1.3 mm i.d. grafts and 867 ± 141 mmHg for 4 mm i.d. grafts. Suture retention strength was a function of the number of layers within the graft wall. The 1.3 mm and 4 mm grafts comprised of four rolled layers (Fig. 2). The suture retention strength was similar for both grafts (30.5 ± 4.2 gF, 1.3 mm i.d. and 37.0 ± 5.1 gF, 4 mm i.d.). Grafts constructed on a 1.3 mm mandrel had a wall thickness of 237 ± 38.6 μ m, an inner diameter of 1.29 ± 0.07 mm and an outer diameter of 1.73 ± 0.08 mm. Grafts produced using 4 mm mandrel had a wall thickness of 223 ± 19.0 μ m, an inner diameter of 4.01 ± 0.02 mm and an outer diameter of 4.49 ± 0.06 mm.

Graft structure and composition

Preliminary studies involving elastin-like proteins coated on ePTFE have demonstrated the hemocompatibility of certain recombinant elastin formulations [37, 38]. In vivo studies

detailed herein utilized grafts with a 1.3 mm i.d. (Fig. 2A, B). Van Gieson staining of collagen fiber containing elastin-like protein matrices illustrates individual ECM components with a luminal coating of the elastin analogue (Fig. 2C). Ultrastructure was characterized by SEM and TEM with a uniform luminal coating of elastin protein polymer (Fig. 2C, D). En face visualization of elastin confirmed its distinct fibrillar structure (Fig 2C, D, E). Collagen matrices demonstrated collagen fibrils with a diameter of 83.1 ± 9.4 nm (Fig. 2F) with D-periodic banding prior to (Fig. 2G) and after embedding within the elastin analogue (Fig. 2H).

Plate adhesion and morphology

Platelet adhesion and activation are one of the key determinants of thrombogenicity. Compared to glass and collagen, ePTFE, ELP, and graft surfaces showed significantly lower platelet adhesion (Fig. 3). Glass had the largest number of adherent platelets ($2.42 \pm 0.10 \times 10^5$ platelets/mm²), followed by collagen ($2.04 \pm 0.09 \times 10^5$ platelets/mm²), ePTFE ($1.62 \pm 0.05 \times 10^5$ platelets/mm²), elastin protein polymer ($6.62 \pm 0.36 \times 10^4$ platelets/mm²), and the luminal surface of protein composite conduits ($6.78 \pm 0.05 \times 10^5$ platelets/mm²). SEM demonstrated a greater number of activated platelets on glass and collagen surfaces than ePTFE with significantly more rounded and sparser platelets noted on elastin and graft surfaces (Fig. 3B–P).

Patent small diameter vascular grafts in vivo

Vascular conduits (1.3 mm i.d.) were implanted in a rat aortic interposition model for 1 – 14 days (Fig. 4A, B). Choice of 275–300g female rats was prompted by approximate size matching of the inner diameter of native vessel. Upon explantation at 14 days, grafts appeared patent with a minimal adhesive response to the abluminal surface. Contrast CT angiography of perfusion fixed interposed grafts showed maintenance of graft patency and lack of aneurysmal dilation (Fig 4. C), which was confirmed by perioperative ultrasound studies (Fig. 4D, E). Visual observation of graft luminal surface showed no thrombus or visible intimal hyperplasia. Graft integrity was maintained with identifiable staining of ECM based graft components (Fig. 5A). Staining of the luminal surface was positive for collagen, mononuclear cells, entrapped red blood cells, and vWF⁺ cells suggestive of an endothelial cell containing neointima (Fig. 5A–C).

DISCUSSION

Collagen and elastin matrices have been used for several decades either independently, in combination or as composites with other materials for tissue engineering [39–41]. Although of great relevance to tissue engineering and the design of materials for implantation, mechanical resistance to failure has hampered efforts to fully utilize the properties of collagen. Several techniques have been developed to aid the localized deposition of collagen on non-biologically derived matrices and to recapitulate the ECM environment using synthetic polymers, or processed collagen. The mechanical strength of traditional processing techniques for collagen matrices do not approximate native tissue, in part, due to denaturation of the triple helical structure and native fibrils. We have generated mechanically tunable and resilient matrices that exhibit high failure strain, through a fabrication technique that promotes compaction of collagen gels and co-gelling with recombinantly expressed elastin. These matrices exhibit mechanical properties that are similar to vascular and other soft tissue. Mechanical strength and stiffness can be varied through the judicious selection of initial gel formulations, concentrations, gel thicknesses, layering of matrices, and crosslinking.

The success of vascular grafts requires compliance matching to native vascular tissue, burst pressures exceeding physiological ranges, and suturability. Compliance mismatch has been indicated in the failure of vascular grafts [42, 43]. Several groups have investigated the use of porous biodegradable matrices that allow cellular infiltration or pre-seeded cells that facilitate remodeling [44–46]. Given the long culture times, risk of contamination, limited autologous cell sources, and high cost, these strategies lack wide-scale clinical applicability [3]. The current trend in the field has been to recapitulate the native blood vessels using in vitro cell- based approaches to secrete ECM that can be used as a scaffold, which is subsequently decellularized [13]. Likewise, long culture times, the potential for contamination, cell sourcing, and cost limits utility. We propose an approach that obviates some of these limitations by rapidly generating protein based vascular grafts using: purified Type I collagen for strength and structural support [29, 47], and recombinant elastin analogues to provide a resilient matrix and a thromboresistant blood contacting surface [29, 37, 38]. Engineered grafts displayed compliance, which approximated that of native vein and adequate suturability. While not as high as native arteries, engineered vessels exhibited burst pressures that were approximately three to four fold greater than maximum physiologic pressures (Table 1) [35]. In this report, the capacity of elastin analogues to undergo a temperature sensitive sol-gel transition provided a means to adhere layers of collagen to each other. Of clinical importance is the long term stability of these tissue engineered vascular grafts and their resistance to delamination. During the 14 day study period we did not note delamination of layers of the rolled grafts. While infiltrating cells were not observed within the media of the construct during this time frame, we did note a small population of CD68⁺ cells and vWF expressing cells on the lumen of graft wall, which may reflect early re-endothelialization of the luminal surface. Long term studies will help determine stability, degradation and remodeling of grafts as a function of cellular infiltrate and protein resorption.

Low platelet adhesion was observed on graft composite and elastin surfaces. Prior studies by our group in a baboon arterio-venous shunt model showed a similar abrogation of platelet adhesion on elastin protein polymer coated ePTFE as compared to uncoated ePTFE [38]. Similarly others have shown the ability for highly hydrophilic or amphiphilic materials to modulate thromboresistance [37, 38]. Compared to recent studies using acellular biodegradable PGS and PLGA scaffolds, we have shown significantly lower platelet adhesion on elastin and graft surfaces [7, 30, 31]. Protein based vascular grafts showed good short term success. Long term studies and implantation within large animal models are ongoing [31].

CONCLUSIONS

We report the production of a protein composite composed of collagen fiber and elastin analogues using a sandwich molding process. We demonstrated the ability to rapidly create rolled tubular constructs that exhibit mechanical properties similar to native blood vessels with limited platelet adhesion and promising short-term performance in vivo. We believe this paradigm represents a promising strategy for engineering tissues with defined extracellular matrix composition, structure, and architecture. Future studies will involve the assessment of grafts in large animal models, seeding of grafts with cells, and incorporation of bioactive molecules to tailor in vivo responses. Positive results with long term studies in rat and larger animals models in the abdominal aorta model will segue applications in dog coronary artery bypass models and in vivo baboon AV shunt models for hemodialysis [7, 35].

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Figure 1. Schematic of fabrication scheme for vascular grafts

(A) Collagen gels are neutralized in buffer at 4°C for 24 h in a rectangular mold (10 × 7 × 0.4 cm), yield 4 mm thick gels. (B) Gels are dried to thin ~40 μm mats and placed on 0.45 μm filter paper (*) atop a fritted filter (\$). (C) Collagen mat on filter paper is placed on a glass slide at 4°C for 30 mins. (D) Green plastic shims are placed around the collagen mat, and elastin, blue, is poured atop with a capping glass slide is placed to spread the elastin, and heated to 25°C. (E) Composite matrices are rolled on 1.3 mm or 4.0 mm mandrels. (F) Rolled matrices are cooled and reheated to allow elastin bonding of collagen layers.

Figure 2. Meso- and ultrastructure of collagen-elastin composite grafts

(A) Photo of unimplanted graft. (B) Long graft segment showing kink resistance. (C) Van Geison stained cross-section of graft wall delineating layers: collagen stained red, elastin yellow (scale bar 100 μm). (D) SEM of graft cross-section (scale bar 100 μm). (E) SEM of fibrous elastin structure on lumen of graft (scale bar 1 μm). (F) SEM of fibrous structure of 2.5 mg/mL collagen matrix showing fibrillar collagen, (50 kX, scale bar 1 μm) with 10 kX inset demonstrating nanofibrous morphology (scale bar 2 μm). (G) TEM of collagen mat showing characteristic collagen banding (scale bar 1 μm). (H) TEM of cross-section of elastin embedded collagen mat showing preservation of native structure, 0.5 μm . *Marks luminal side.

Figure 3. Platelet adhesion quantification and morphology

(A) Number of platelets on various surfaces quantified using LDH cytotoxicity assay, and compared to known platelet concentrations. Glass surfaces showed greatest number of adhered platelets, compared to collagen, which was greater than ePTFE which was in turn greater than elastin and graft samples. Number of platelets adhered to elastin and graft surfaces were not significantly different. (B, E, H, K, N) 2 kX magnification image of platelets on glass, collagen, ePTFE, elastin and graft surfaces respectively, showing qualitatively global distribution of platelets. (C, F, I, L, O) 6 kX magnification image of platelets on glass, collagen, ePTFE, elastin and graft surfaces respectively, showing qualitatively regional distribution and morphology of platelets. (D, G, J, M, P) 18 kX magnification image of platelets on glass, collagen, ePTFE, elastin and graft surfaces respectively, showing morphology of adhered platelets (n = 4 for each sample, * $p < 0.01$).

Figure 4. Implantation of an aortic interposition graft in the infrarenal position

Gross morphology of the graft (1cm length, 1.3 mm ID): **(A)** Photo of graft at implant showing reddish color of blood flow. **(B)** Photo of graft after explantation, exsanguination, and perfusion fixation. **(C)** Evaluation of graft patency using CT angiography. **(D, E)** Evaluation of graft prior to explantation using color Doppler ultrasound and Doppler velocity distribution.

Figure 5. Histological evaluation of early biological responses to implanted composite grafts

Native distal aorta (**A–D**) and composite graft (**E–H**) at 2 week time point. Hemotoxylin and eosin staining shows minimal cellular infiltration into aorta (**A**) or graft wall (**E**). Masson's Trichrome demonstrates cellular intima (pink/purple) and medial connective tissue/collagen (blue) of host vessel (**B**) and graft (**F**). vWF staining of lumen of native vessel (**C**) and graft (**G**). CD68 staining for macrophages shows limited macrophage infiltration in native vessel (**D**) or graft (**H**).

Table 1

Mechanical Characterization of Composite Vascular Grafts

	Wall thickness (μm)	Compliance (%/100 mmHg)	Burst Pressure (mmHg)	Suture retention strength (gF)
Implant Graft 1.3 mm	237 $\pm$ 38.6	2.7 $\pm$ 0.3	830 $\pm$ 131	30.5 $\pm$ 4.2
Implant Graft 4.0 mm	223 $\pm$ 19.0	2.9 $\pm$ 0.2	867 $\pm$ 141	37.0 $\pm$ 5.1
Venous - Saphenous, Umbilical*	250	0.7 - 3.7	1600 - 2500	180 - 250
Arterial - ITA, Coronary, Carotid, Femoral, Popliteal*	350-710	4.7 - 17.0	2200 - 4225	88 - 200
Commercial synthetic grafts*	200-600	0.2 - 1.9	2580 - 8270	250 - 1200